fibers, discard the first 10 mL of the filtrate, and use the subsequent filtrate as the sample solution. Separately, dry Reserpine Reference Standard at 60°C in vacuum for 3 hours, weigh accurately an amount 100 times the labeled amount, dissolve in 1 mL of chloroform and 80 mL of ethanol (95), and add a solution of polysorbate 80 in diluted dilute acetic acid (1 in 200) (1 in 1000) to make exactly 200 mL. Pipet 1 mL of this solution, add a solution of polysorbate 80 in diluted dilute acetic acid (1 in 200) (1 in 1000) to make exactly 250 mL, and use this solution as the standard solution. Pipet 5 mL each of the sample solution and the standard solution, transfer to glass-stoppered brown test tubes T and S, respectively, add exactly 5 mL each of ethanol (99.5), shake well, add exactly 1 mL each of diluted vanadium (V) oxide (1 in 2), shake vigorously, and allow to stand for 30 minutes. Perform the test with these solutions as directed under the Fluoroscopy, and determine the intensity of fluorescence, F_T and F_S, at the wavelength of excitation at 400 nm and at the wavelength of fluorescence at 500 nm.

Dissolution rate of Reserpine Tablets after 30 minutes should be not less than 70%.

$$\text{Dissolution rate (\%) to the labeled amount of reserpine } (C_{33}H_{40}N_2O_9) = W_S \times \frac{F_T}{F_S} \times \frac{1}{C}$$

W_S: Amount (mg) of Reserpine Reference Standard.
C: Labeled amount (mg) of reserpine ($C_{33}H_{40}N_2O_9$) in each tablet.

Content uniformity Conduct this procedure without exposure to daylight, using light-resistant vessels. To one tablet of Reserpine Tablets add 2 mL of water, disintegrate by warming at 50°C for 15 minutes while shaking. After cooling, add exactly 2 mL of the internal standard solution per 0.1 mg of reserpine according to the labeled amount, add 2 mL of acetonitrile, warm at 50°C for 15 minutes while shaking, and after cooling, add water to make 10 mL. Centrifuge the solution, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.01 g of Reserpine Reference Standard, previously dried at 60°C in vacuum for 3 hours, dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Proceed with the sample solution and the standard solution as directed in the Assay under Reserpine.

$$\text{Amount (mg) of reserpine } (C_{33}H_{40}N_2O_9) = \text{amount (mg) of Reserpine Reference Standard} \times \frac{Q_T}{Q_S} \times \frac{\text{labeled amount (mg) of reserpine in each tablet}}{10}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

Assay Conduct this procedure without exposure to daylight, using light-resistant vessels. Weigh accurately and powder not less than 20 Reserpine Tablets. Weigh accurately a quantity of the powder, equivalent to about 0.5 mg of reserpine ($C_{33}H_{40}N_2O_9$), add 3 mL of water, and warm at 50°C for 15 minutes while shaking. After cooling, add exactly 10 mL of the internal standard solution, 10 mL of acetonitrile and warm at 50°C for 15 minutes while shaking. After cooling, add water to make 50 mL, centrifuge, and use the supernatant liquid as the sample solution. Separately, weigh accurately about 0.01 g of Reserpine Reference Standard, previously dried at 60°C in vacuum for 3 hours, and dissolve in acetonitrile to make exactly 100 mL. Pipet 5 mL of this solution, add exactly 10 mL of the internal standard solution, 5 mL of acetonitrile and water to make 50 mL, and use this solution as the standard solution. Proceed with the sample solution and the standard solution as directed in the Assay under Reserpine.

$$\text{Amount (mg) of reserpine } (C_{33}H_{40}NO_9) = \text{amount (mg) of Reserpine Reference Standard} \times \frac{Q_T}{Q_S} \times \frac{1}{20}$$

Internal standard solution—A solution of butyl parahydroxybenzoate in acetonitrile (1 in 50,000).

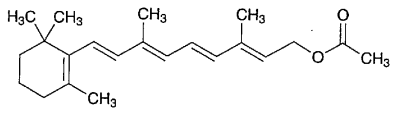
Containers and storage Containers—Well-closed containers.

Storage—Light-resistant.

Retinol Acetate

Vitamin A Acetate

酢酸レチノール



$C_{22}H_{32}O_2$: 328.49
(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl acetate [*127-47-9*]

Retinol Acetate is synthetic retinol acetate or synthetic retinol acetate diluted with fixed oil. It contains not less than 2,500,000 Vitamin A Units per gram. A suitable antioxidant may be added. Retinol Acetate contains not less than 95% and not more than 105% of the labeled Units.

Description Retinol Acetate occurs as pale yellow to yellow-red crystals or an ointment-like substance, and has a faint, characteristic odor, but has no rancid odor.

When powdered, it is very soluble in chloroform and in diethyl ether, freely soluble in petroleum diethyl, soluble in 2-propanol and in ethanol (95), and practically insoluble in water.

It is affected by air and by light.

Identification **(1)** Prepare a solution of Retinol Acetate in chloroform containing 30 Vitamin A Units per mL according to the labeled Units, pipet 1 mL of the solution, and add 3 mL of antimony (III) chloride TS: a blue color develops immediately, then fades rapidly.

(2) Proceed with Retinol Acetate as directed in the Identification, Method 1 under the Vitamin A Assay, and perform the test: the color tone and the *Rf* value of the main spot from the sample solution correspond to those of the blue spot from retinol acetate from the standard solution, and no spot appears from the sample solution having the

same color tone and the *Rf* value as those of the blue spot from retinol palmitate from the standard solution.

Purity Related substances—Retinol Acetate meets the requirements of Method 1 under the Vitamin A Assay.

Assay Proceed as directed in Method 1 under the Vitamin A Assay.

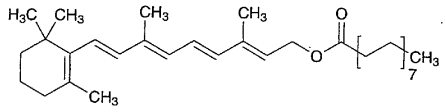
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under nitrogen atmosphere, and in a cold place.

Retinol Palmitate

Vitamin A Palmitate

パルミチン酸レチノール



$C_{36}H_{60}O_2$: 524.86

(2*E*,4*E*,6*E*,8*E*)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohex-1-en-1-yl)nona-2,4,6,8-tetraen-1-yl palmitate [*79-81-2*]

Retinol Palmitate is a synthetic retinol palmitate or a synthetic retinol palmitate diluted with fixed oil, and contains not less than 1,500,000 Vitamin A Units in each gram. It may contain a suitable antioxidant.

Retinol Palmitate contains not less than 95% and not more than 105% of the labeled Units.

Description Retinol Palmitate occurs as a light yellow to yellow-red, ointment-like or an oily substance. It has a faint, characteristic odor, but has no rancid odor.

It is very soluble in 2-propanol, in chloroform, in diethyl ether and in petroleum ether, slightly soluble in ethanol (95), and practically insoluble in water.

It is affected by air and by light.

Identification **(1)** Prepare a solution of Retinol Palmitate in chloroform containing 30 Vitamin A Units per mL according to the labeled Units, pipet 1 mL of the solution, and add 3 mL of antimony (III) chloride TS: a blue color develops immediately, then fades rapidly.

(2) Proceed with Retinol Palmitate as directed in the Identification, Method 1 under the Vitamin A Assay, and perform the test: the color tone and the *Rf* value of the main spot correspond to those of the blue spot from retinol palmitate from the standard solution, and no spot appears from the sample solution having the same color tone and the *Rf* value as those of the blue spot from retinol acetate from the standard solution.

Purity Related substances—Retinol palmitate meets the requirements of Method 1 under the Vitamin A Assay.

Assay Proceed as directed in Method 1 under the Vitamin A Assay.

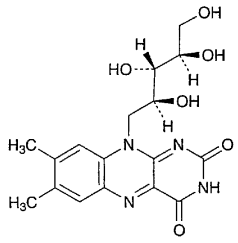
Containers and storage Containers—Tight containers.

Storage—Light-resistant, and almost well-filled, or under nitrogen atmosphere, and in a cold place.

Riboflavin

Vitamin B_2

リボフラビン



$C_{17}H_{20}N_4O_6$: 376.36

7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]-benzo[*g*]pteridine-2,4(3*H*,10*H*)-dione [*83-88-5*]

Riboflavin, when dried, contains not less than 98.0% of $C_{17}H_{20}N_4O_6$.

Description Riboflavin occurs as yellow to orange-yellow crystals. It has a slight odor.

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

It dissolves in sodium hydroxide TS.

A saturated solution of Riboflavin is neutral.

It is decomposed by light.

Melting point: about 290°C (with decomposition).

Identification **(1)** A solution of Riboflavin (1 in 100,000) is light yellow-green in color and has an intense yellow-green fluorescence. The color and fluorescence of the solution disappear upon the addition of 0.02 g of sodium hydrosulfite to 5 mL of the solution, and reappear on shaking the mixture in air. This fluorescence disappears upon the addition of dilute hydrochloric acid or sodium hydroxide TS.

(2) To 10 mL of a solution of Riboflavin (1 in 100,000) placed in a glass-stoppered test tube add 1 mL of sodium hydroxide TS, and after illumination with a fluorescence lamp of 10 to 30 watts at 20-cm distance for 30 minutes between 20°C and 40°C, acidify with 0.5 mL of acetic acid (31), and shake with 5 mL of chloroform: the chloroform layer shows a yellow-green fluorescence.

(3) Determine the absorption spectrum of a solution of Riboflavin in phosphate buffer solution, pH 7.0 (1 in 100,000) as directed under the Ultraviolet-visible Spectrophotometry, and compare the spectrum with the Reference Spectrum or the spectrum of a solution of Rivoflavin Reference Standard prepared in the same manner as the sample solution: both spectra exhibit similar intensities of absorption at the same wavelengths.

Optical rotation $[\alpha]_D^{20}$: -128 – $-142°$ Weigh accurately about 0.1 g of dried Riboflavin, dissolve in exactly 4 mL of dilute sodium hydroxide TS, add 10 mL of freshly boiled and cooled water, add exactly 4 mL of aldehyde-free alcohol while shaking, add freshly boiled and cooled water to make exactly 20 mL, and determine the rotation in a 100-mm cell within 30 minutes after preparing the solution.

Purity Lumiflavin—Shake 0.025 g of Riboflavin with 10 mL of ethanol-free chloroform for 5 minutes, and filter: the